

Application. No. 09/977,137
Amendment dated September 16, 2003
Reply to Office Action of June 17, 2003

Amendments to the Specification:

Please replace the paragraph beginning at page 1, line 2, with the following amended paragraph:

—This application is a continuation-in-part claims benefit of United States Provisional Application No. 60/240,465, filed October 12, 2000, which is incorporated herein to the extent that there is no inconsistency with the present disclosure.—

Replace the paragraph beginning at page 14, line 5, with the following amended paragraph:

—The complete nucleotide sequence encoding the MerR protein of Tn21 is available on GenBank, Accession No. P07044. Plasmid pASK-IBA3 is commercially available from Sigma-Genosys (The Woodlands, TX). The StrepTagII (trademark of Institut fur Bioanalytik GmbH) technology (which depends on streptavidin binding by particular residues, i.e., WSHPQFEK amino acid residues present at the C-terminus of the recombinant chelon protein; amino acids 110-117 of SEQ ID NO:4) is described in United States Patent No. 5,506,121, incorporated by reference herein. The recombinant mercury-binding chelon expression plasmid is constructed by joining PCR amplicands of two copies of sequences encoding the metal binding domain (residues 81-127) of MerR. To facilitate joining of these 2 metal binding domains coding sequences in a direct tandem repeat, the primers used in the amplification are designed to have a common BamHI restriction endonuclease recognition site to allow the to be joined and also to include a linker regions between them (See Fig. 4). The outer primers add a BsaI site to each end, allowing the entrc entrc chelon coding sequence to be cloned into the vector pASK-IBA3 (at the BsaI site remaining after the short BsaI fragment is removed and both BsaI fragments were eliminated). The expression of the tagged protein is under the control of the tetracycline-inducible *tetA*

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promoter, and the polylinker into which a coding sequence of interest is cloned contains restriction endonuclease recognition sites for *Bsa*I and *Bsm*FI. Crude extracts of the recombinant *E. coli* cells containing the tagged chelon protein are treated to purify the tagged protein in accordance with instructions from the manufacturer.—

Replace the header for Table 1C beginning at page 20, line 42, with the following amended header:

—Complete amino acid sequence of the chelon protein (SEQ ID NO:4) showing novel residues not found in wild-type MerR. The last underlined residues are derived from the StrepTag vector (Genosys) and are not essential to the metal-binding domain nor do they interfere with metal binding (SEQ ID NO:3). These residues are only important for purification of the protein.—